

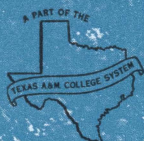
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JULY 1960

THE VITASCOPE

An Aid For
Rapid Determination
of Viable Seed
with Tetrazolium

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Summary

Tetrazolium (triphenyl tetrazolium chloride) is a colorless solution. However, when in contact with live embryo tissue of seed, the embryo stains red. The germination and vigor of seed may be estimated by this method. A tetrazolium staining device called the "vitascope" accelerates the staining process and the germination may be estimated in 10 to 50 minutes. The conventional tetrazolium stain method requires a staining period of 4 to 48 hours, depending on the type of seed tested.

Dicotyledonous seed such as cotton, field peas and soybeans were considered capable of germination if the primary root, hypocotyl and point of epicotyl attachment have a positive red stain. If one-fourth or more of the cotyledonous area remained colorless after staining, the seed were considered weak or nongerminable.

Monocotyledonous seed such as corn, sorghum and small grains were considered capable of ger-

mination if all of the primary root, plumule and mesocotyl region and one-fourth to one-half of the scutellum region stained a positive red. The primary root may remain colorless in monocotyledonous seed such as corn and small grains, but the seed still be capable of germination provided the secondary root buds are stained indicating viability.

The most common type of staining pattern exhibited by monocotyledonous seed low in vigor was a mottled or lightly stained scutellum with the remaining embryo structures staining a positive red.

Seed that were made nonviable by methyl bromide fumigation indicated a positive red stain in the tetrazolium test.

The potential germination levels of partially dormant seed of several grass and clover species were indicated by the tetrazolium tests.

Definition of Terms

Cotyledon—The first leaves on the embryo, one in monocotyledonous seed, two or more in dicotyledonous seed.

Epicotyl—That portion of an embryo or seedling above the cotyledons; plumule.

Hypocotyl—The part of the embryo or seedling below the cotyledon and above the root; the transition region that connects the stem and root.

Plumule—The embryonic leaves of the embryo (epicotyl) surrounded by a protective sheath or coleoptile.

Primary root—The rudimentary root of the embryo; radicle.

Scutellum—The cotyledon of an embryo of such seed as corn, sorghum, oats, wheat, etc.; a food absorbing structure.

Mesocotyl—The region below the node of the plumule and its protective sheath; collectively called the coleoptilar node.

Dicotyledonous seed—A seed having two cotyledons; example, cotton, field peas and soybeans.

Monocotyledonous seed—A seed having one cotyledon (scutellum); example, corn, sorghum and small grains.

Dormant seed—A delayed germination of growth of viable seed due to such conditions as impermeable seed coats, immature embryos and inhibiting substances.

Embryo—The rudimentary plant formed in a seed; the germ.

Endosperm—The reserve, stored food of seed outside the embryo.

Germinable—Possessing a germination potential.

Germination—The resumption of active growth by the embryo in a seed.

Viability—The ability to live, grow and develop.

Vigor—The strength or force of seedling and plant growth.

Abnormal seedling—A seedling with weak or malformed structures such as stubby roots, split hypocotyl, absence of plumule; not potentially capable of producing a normal plant under favorable growing conditions.

Normal seedling—A seedling with healthy, well-formed structures such as epicotyl, hypocotyl and root; potentially capable of producing a healthy plant under favorable growing conditions.

The Vitascope-An Aid for Rapid Determination of Viable Seed with Tetrazolium

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LAKON (1949) WAS ONE OF THE FIRST WORKERS to use tetrazolium (2,3,5 triphenyl tetrazolium chloride) to estimate the germination of seed. Since that time several other research workers have used this chemical to estimate seed germination. Tetrazolium in water is a colorless solution which changes to a red stain (red triphenyl formazan) in the presence of live embryo tissue of seed. Dead (nonviable) seed remain colorless in tetrazolium or stain only lightly. Moore (1956) observed that bruised or damaged seed tissue of recent occurrence will develop rapidly a dark red stain in the tetrazolium test while nonbruised tissue, if alive, gradually develops a uniform, clear red color. If bruised or mechanically damaged seed are stored under conditions that cause a rapid decline in germination, these damaged areas remain colorless after exposure to tetrazolium solution.

The tetrazolium stain test is being used by many seedsmen because of the rapidity of the test. For example, some of the cotton delinting or seed processing plants use the stain test to determine in a few minutes the quality of seed prior to delinting or processing. This information allows the operator to reject instantly any seed lots that are low in germination.

Internal seed weaknesses such as dead parts of the embryo and mechanically bruised areas are revealed by the stain test. Injured seed of this type still may germinate, but in most instances the seedlings will be low in vigor. This additional information concerning seed vigor is very valuable, particularly if the seed are planted in a cold soil or held in storage until the following year.

Considerable time is required by the standard laboratory germination method to determine the number of nonviable seed or abnormal seedlings. This laboratory germination period may vary from 5 to 35 days depending on the crop tested. Although the standard germination test is desirable for obtaining accurate information on the ability of seed to develop seedlings under optimum conditions, in many instances it furnishes little or no information on weakened internal seed structures. Poor stands may be obtained from weak seed under adverse planting conditions. The percent emergence of seed under ad-

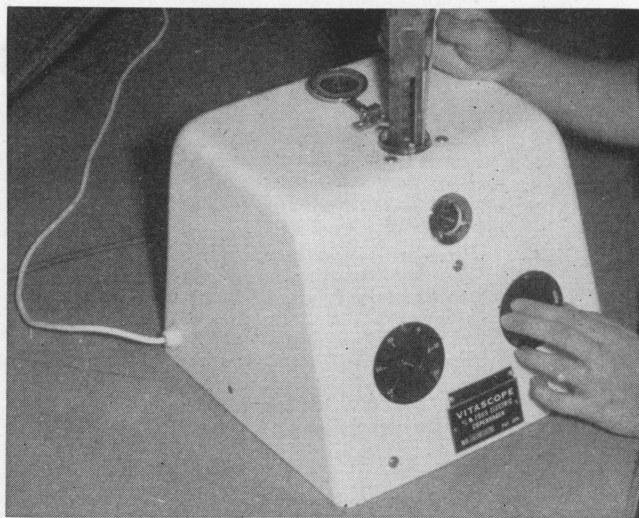


Figure 1. A sample of seed is being placed in the vitascope reaction chamber for staining.

verse planting conditions may be determined by a cold test; however, this method is expensive and time consuming. A staining device called a "vitascope" recently brought renewed interest to the tetrazolium stain test, Figure 1. The vitascope accelerates the staining process by subjecting the seed to a partial vacuum and to heat during exposure to tetrazolium solution.

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TABLE 1. THE PREPARATION OF SEED OF VARIOUS CROPS FOR VITASCOPE TESTING AND THE INTERPRETATION OF GERMINABLE SEED BY TETRAZOLIUM STAINED EMBRYO STRUCTURES

Crop	Seed preparation before staining in vitascope ¹	Staining period	Interpretation of germinable seed by stained embryo structures ²	Agreement with standard germination ³
Corn	Kernels are allowed to soften in a container of warm (86° F.) tap water for approximately 4 to 6 hours. A wetting agent is added to tap water for softening extremely dry seeds. After the softening period, the seed embryo is bisected longitudinally and placed in vitascope for staining.	10 to 15 minutes	Seed considered germinable if plumule and mesocotyl region stains red and at least one half to three fourths of scutellum reveals a red coloration. The primary root can remain colorless provided the above structures are viable.	Good — relatively large size of embryo facilitates interpretation of stained structures.
Sorghum	Preparation same as for corn, except the softening period is frequently less than for corn, usually 2 to 3 hours. Softening facilitates the bisecting process. After softening sudan, the glumes are removed before bisecting and staining.	10 minutes	The embryo proper which includes the plumule, mesocotyl and primary root (radicle) must stain red and one half to three fourths of the scutellum. Faint or light red-stained scutellum indicates weak seed still capable of germinating.	Good — dissecting microscope aids interpretation of stained embryo structures.
Small Grain Oats Barley Wheat	Presoftern in warm water and wetting agent for approximately 4 hours. Seed embryo bisected longitudinally with single edge razor blade. Dehulling oats is desirable before bisecting, but this technique requires considerable time.	10 to 15 minutes	The protruding portion of the lower embryo is susceptible to mechanical bruises which the stain test frequently shows as colorless tissue. Seed are considered germinable if the colorless area has not invaded the mesocotyl region and upper regions of the embryo (plumule).	Good — in freshly harvested seed, stain test slightly overestimates actual germination.
Cotton	Soften in container of warm tap water (86° F.) and wetting agent for approximately 6 hours. After softening, seed coat is removed before staining. Hard seed are bisected through the primary root.	15 to 20 minutes	Primary root (radicle) and point of epicotyl attachment must stain red to be considered germinable. Small colorless areas on the cotyledon are allowable, provided the root and hypocotyl structures are viable. Seed revealing colorless areas on cotyledon will frequently develop weak seedlings.	Good — in highly vigorous seed germinating 85 to 90 percent. Fair to poor agreement on low quality seed with excessive deterioration. Stain test frequently overestimates actual germination of low quality cotton seed.
Field peas Soybeans Beans, etc.	Seed placed between moist paper toweling 1 to 2 hours and then placed in beaker of tap water and wetting agent for 2 to 3 hours. Rapid absorption of water causes fracturing of these seed which necessitates the slow presoftering in moist toweling. After softening, the seed coat is removed prior to staining.	15 to 20 minutes	Interpretation same as for cotton seed. Protruding root and hypocotyl tissue frequently become weak or completely dead due to mechanical damage during harvesting and processing.	Good — provided careful attention is given each sample to maintain optimum germination conditions.
Grass seed (not including corn, sorghum, wheat, etc.)	Grass seed placed between moist paper toweling overnight. Species of grass seed too small for bisecting were punctured with a needle through the seed coat near the region of the embryo. The lemma and palea were removed in the relatively larger grasses such as Rescue and Western Wheat grass prior to bisecting and staining.	35 to 50 minutes	Considered germinable if one half to three fourths of the embryo stains red which must include the plumule and mesocotyl region with at least a portion of the scutellum region. Extremely small size of some grass seed embryos offers a problem in detecting the stained embryo structures.	Fair — stain test frequently overestimates actual germination. In samples exhibiting partial dormancy, a closer agreement is obtained if dormancy is removed prior to germination.

¹All seed samples prepared for vitascope testing were either bisected or some type of opening provided for entrance of tetrazolium solution into the embryo.

²A faint red stain or off-type coloration usually indicates nongerminable seed or seed low in vigor.

³Comparison of both viability test methods was made on seed stored under normal conditions at the Foundation Seedstock Building, College Station, Texas.

Several thousand seedlots of various crops have been stained with the aid of the vitascope. A duplicate seed sample was germinated to compare the results of the stain method with the conventional germination method. The objective of this paper is to furnish information to seedsmen and seed analysts to aid them in conducting the tetrazolium test and in interpreting the results.

Seed Preparation For Tetrazolium Testing

The seed must be prepared carefully before it is stained if accurate estimates of germination and seed weaknesses are to be detected. The seed should be soaked in warm water and the embryo of the seed bisected or its seed coat removed prior to staining. Seed such as clover and grass may be stained with a broken or punctured seed coat.

Presoaking the seed in water accelerates respiration, initiates germination and often is necessary to obtain a desirable stain. In addition, the softened seed are more easily bisected or dehulled after presoaking, and the entrance of tetrazolium is facilitated. Freshly harvested seed with a high moisture content frequently require a shorter period of presoaking. Exceptionally dry or hard seed require a longer period of presoaking; however, if a wetting agent such as Tween 20 is added to the water, the time required for seed softening is decreased considerably. Seed of some crops such as sorghum may stain a bright red with little or no presoaking in water. However, hard unsoaked seed are difficult to bisect through the embryo.

After the required period of soaking, Table 1, in either a beaker of water or moist toweling, seed such as corn, sorghum and small grains should be bisected longitudinally through the embryo (germ). A single edge razor blade or scalpel, Figure 2, is satisfactory for this purpose. Longitudinally bisected seed of various crops in which each embryo half is left intact for staining is shown in Figure 3. The seed coat usually must be removed from dicotyledonous seed (seed containing two cotyledons) such as cotton, peas and soybeans to allow the internal structures of the seed to be in direct contact with the tetrazolium, Figure 4. Clover seed will stain satisfactorily if the seed coat is broken or punctured so that the tetrazolium solution can enter. Grass seed too small for bisecting can be stained properly if the seed coat is punctured above or near the embryo region. Some type of magnification is helpful to insure a uniform opening and proper interpretation of grass species.

Dicotyledonous seed often retain portions of the seed coat which results in uneven staining and difficulty in interpretation. Rapid absorption of water by dicotyledonous seed frequently causes the cotyledons to fracture and the operator may mistake them for bruises and mechanical damage caused by harvesting or processing.

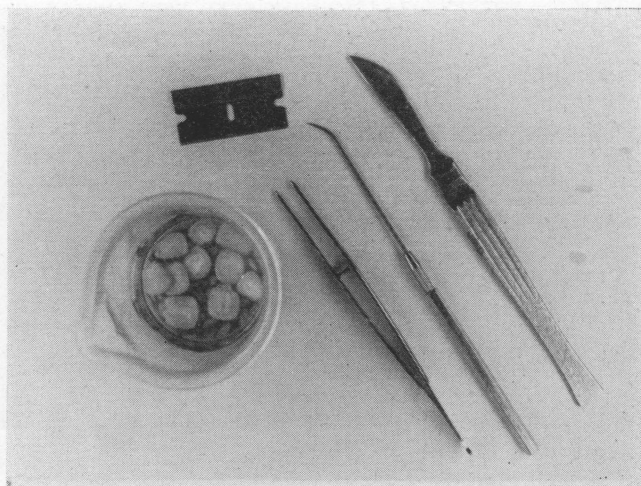


Figure 2. Instruments used in preparing seed for the tetrazolium stain test. Scalpel or razor blade used for bisecting seed. A needle and forceps for transferring it. Seed presoaked in a beaker of water.

The operator should be aware of any seed injuries, such as bruises and missing embryo parts, occurring during the seed preparation prior to staining. The methods of preparing seed of various crops for staining in the vitascope are presented in Table 1.

Conventional and Vitascope Stain Methods

The conventional tetrazolium stain test is conducted by allowing the prepared seed to remain in a 0.5 percent to 2 percent water solution of tetrazolium. The period of staining varies from 4 to 48 hours depending on the temperature, seed preparation and kind of seed tested. Frequently, seed receiving insufficient presoaking in water will require longer periods of staining in tetrazolium. In general, small grass seed such as Rescue and Weeping Love require longer periods of staining than the larger type grass seed (corn,

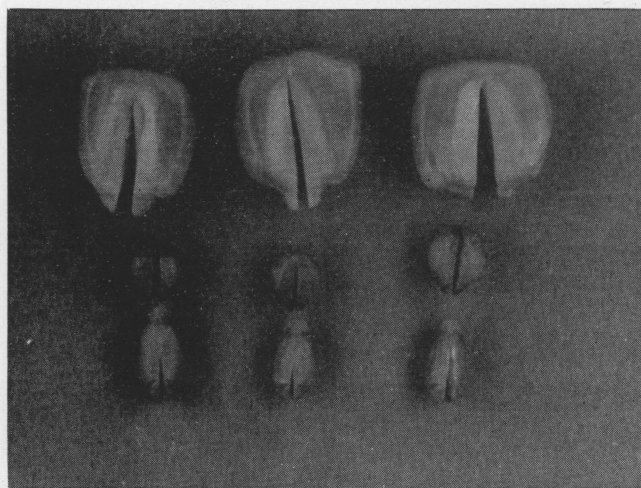


Figure 3. Presoaked seed of corn, sorghum and wheat bisected longitudinally through the embryo.

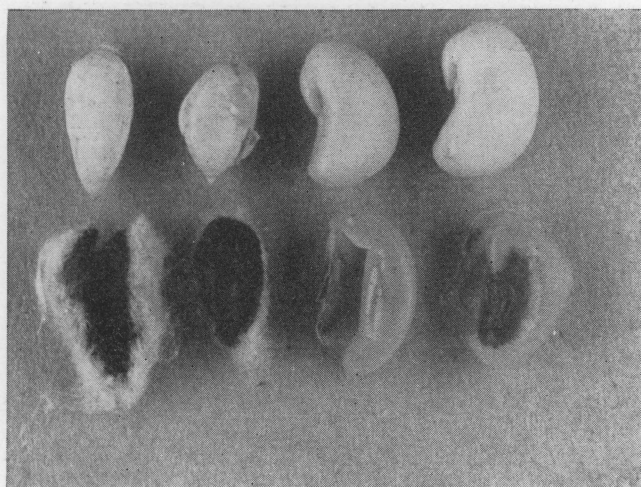


Figure 4. Seed coat removed from cotton (left) and field peas (right) before staining.

sorghum, small grains). However, the operator can terminate the staining period any time the degree of coloration is sufficient for interpretation.

The relatively new vitascope method for tetrazolium testing produces a positive red stain on viable seed in 10 to 50 minutes. Like the conventional method, the length of time for staining in the vitascope is determined by previous seed preparation and the kind of seed tested. The vitascope has a reserve container for tetrazolium and a reaction chamber in which the seed are placed for staining. Both chambers are connected by a spiral copper coil which is thermostatically maintained at 45° C. with a heat bulb. When the seed reaction chamber is placed under

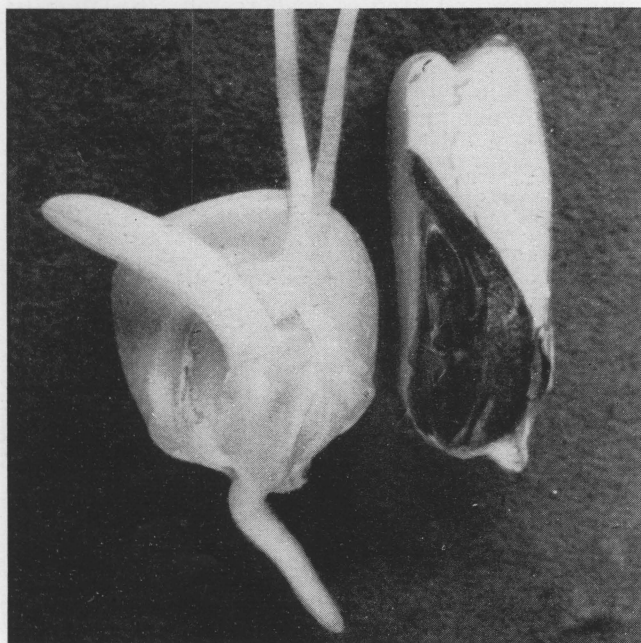


Figure 5. Two kernels of corn taken from the same sample. Right, vigorous seedling; left, positive-stained corn embryo.

vacuum by a pump connected to a standard water outlet, the solution will flow out of the reserve container into the reaction chamber. The seed in the reaction chamber will be under a partial vacuum while exposed to tetrazolium at 45° C. The stain reaction is accelerated by the partial vacuum and high temperature conditions in the vitascope. The partial vacuum causes the tetrazolium to move rapidly into the embryo tissue. The length of the staining period is controlled by a timing device which releases the vacuum and allows the tetrazolium solution to flow back into the reserve container at the end of the test period.

Stain tests conducted by either the conventional or vitascope methods require essentially the same seed preparation, although longer periods of presoaking in water frequently are necessary for staining seed by the conventional method. A 0.5 percent to 2 percent water solution of tetrazolium is recommended for either staining method, but the results so far do not indicate that the 2 percent solution is superior to the 0.5 percent solution.

The powder form of tetrazolium can be purchased from most leading chemical companies for approximately 30 to 33 cents per gram. A 0.5 percent water solution can be prepared by dissolving 2 grams of tetrazolium in a pint of water. To make up this solution, distilled water is preferable, but it is not essential. The solution must be stored in a dark bottle, preferably in a refrigerator, since tetrazolium is unstable in light. The seed must be kept in total darkness during the staining process if the conventional stain method is used. The brief exposure to light in a vitascope allows repeated use of the solution; however, the continuous use of the solution will cause dilution which may cause a slow, weak stain reaction. Frequent changes of tetrazolium are necessary when testing weak seed, especially deteriorated oil seed such as cotton and peanuts.

If time does not permit immediate interpretation of stained seed by either method, the seed may be placed in a container of water and kept in the refrigerator for later interpretation for a period not longer than 2 or 3 days. If stained seed are kept for longer periods, the red stain (triphenyl formazan) eventually will spread to all portions of the seed.

Interpreting Tetrazolium Stain Test

The presence or absence of stained embryo parts is the basis for determining germinable seed with tetrazolium. If the embryo is alive it will stain a carmine red after being exposed to colorless tetrazolium, Figure 5. Those parts of the embryo that remain colorless or only faintly red after staining are considered nonviable, Figure 6. The germination is estimated by the intensity of the stain coloration and the embryo parts that



Figure 6. Nongerminating corn seed indicated by germination test (left) and tetrazolium test (right). Note the colorless embryo parts in the stained kernel.

stain. The methods for interpreting germinable seed of various crops with tetrazolium are presented in Table 1. This method of estimating the germination of seed is not accurate if the seed have been killed by fumigation with methyl bromide. All tetrazolium tests have overestimated the potential germination of seed injured by methyl bromide. This fumigant in some manner kills or inhibits the growth mechanism, but it also apparently alters or produces some substances capable of changing tetrazolium to a red stain (triphenyl formazan).

To interpret accurately the tetrazolium stain test, the operator must be familiar with various seed embryo parts and their subsequent development into seedlings.

The comparative structures of the two types of seed, monocotyledonous and dicotyledonous, are shown in Figure 7. Either type of seed may

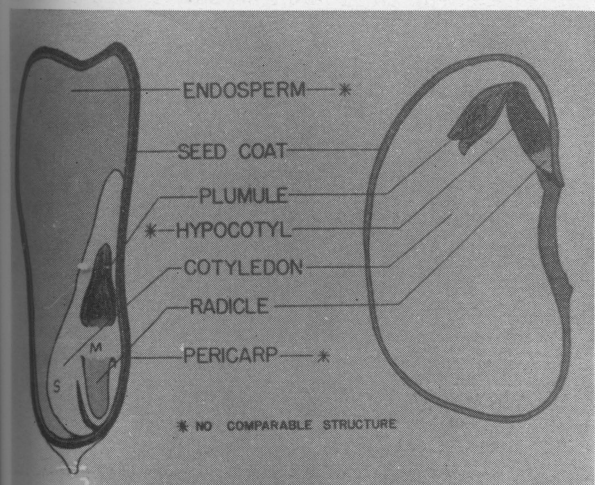


Figure 7. Comparable structures of two types of seed. Monocotyledonous seed (left, corn) and dicotyledonous type seed (right, field peas). M. Mesocotyl region. S. Scutellum (cotyledon).

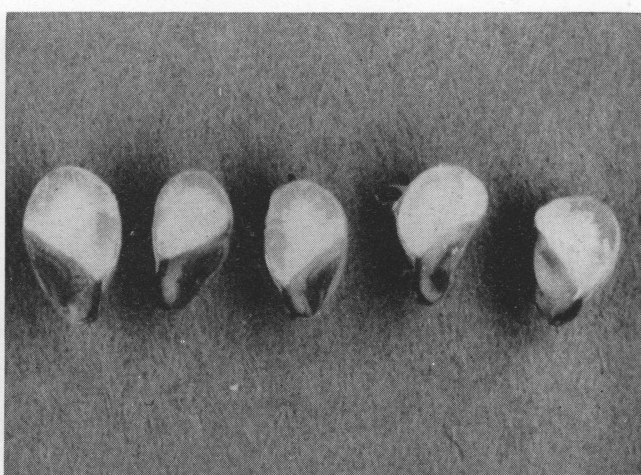


Figure 8. Sorghum seed mechanically damaged. Left to right: First two seeds germinable; the remaining three are nongerminable. Note the dead, colorless tissue in the protruding portion of the embryo.

be considered as a miniature plant (embryo or germ) with all the essential parts enclosed in a package (seed coat) with a built-in food supply (cotyledon or endosperm). When these seed are exposed to favorable germinating conditions, the embryo parts use the built-in food supply for resumed growth and development until the elongated embryo parts (seedling) are capable of producing their own food. Thus, in both types of seed there are certain critical embryo parts that must be alive in order to develop normal seedlings.

In seed of monocotyledonous plants such as corn, wheat, sorghum and oats the plumule and its protective sheath must be alive to offer protection to young embryonic leaves as they emerge through the soil. If the scutellum region has large areas of dead tissue, the supply of food may not be transferred from the endosperm to the growing embryo parts. The seed of monocoty-

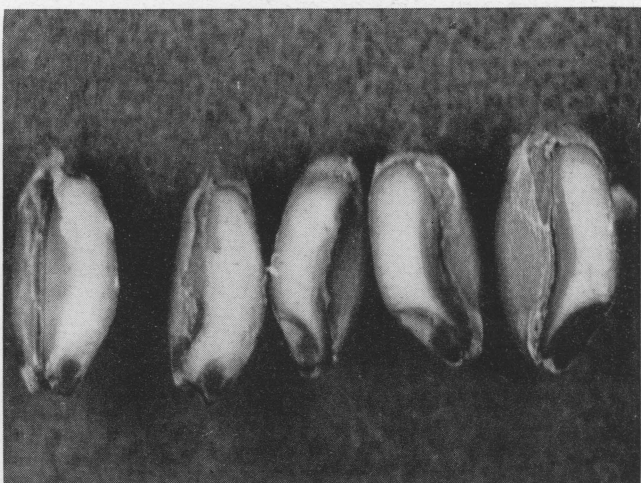


Figure 9. Wheat seed. Tetrazolium test indicates dead, colorless plumules. Seed on extreme right reveals normal stain reaction.

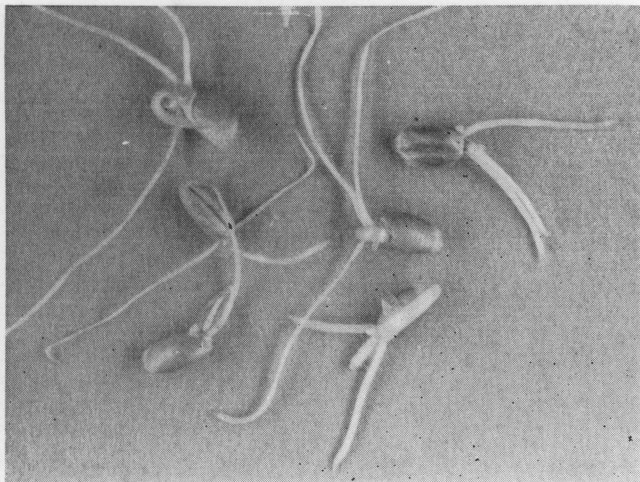


Figure 10. Wheat seedlings produced from seed with dead plumule tissue. Note the abnormal or complete absence of plumules (shoots) while root growth appears normal.

ledonous plants are considered germinable by the stain method if the plumule, primary root, scutellum and mesocotyl region stain red; however, in corn seed the primary root may remain colorless (dead), but it is considered germinable if the upper secondary root buds reveal a positive red stain. Seed having a weak or dead primary root often emerge slowly under adverse conditions in the field. Sorghum seed have a protruding lower scutellum and primary root which is susceptible to mechanical bruises during harvesting and processing. The protruding embryo portions in sorghum are frequently colorless in stain tests; however, the bruised tissue usually is not damaged enough to prevent all seed from germinating, Figure 8. Seed of small grains with dead tissue in the lower primary root area may not germinate if the dead, colorless tissue includes the more vital upper primary root and mesocotyl region. Wheat seed with colorless plumules in stain test

often will produce seedlings that have normal roots, but no plumule or shoot growth if the seed are germinated, Figures 9 and 10.

The seed of dicotyledonous plants have essential embryo parts such as the primary root, hypocotyl and point of epicotyl attachment to the cotyledons that must possess live tissue for normal seedling development. Any mechanical impact upon these areas during harvesting or processing may cause the seed to become nonviable. Small dead areas on the cotyledons of dicotyledonous seed usually will not prevent germination if the tissue in the primary root, hypocotyl and epicotyl attachments are viable. In tetrazolium tests the seed of such crops as cotton, soybeans, peas and beans should not be considered germinable unless the primary root, hypocotyl and point of epicotyl attachment to the cotyledons stain a positive red. These seed often will have numerous colorless areas over the cotyledons when exposed to tetrazolium. If one-fourth or more of the area on the cotyledons is colorless, the seed may produce extremely weak or abnormal seedlings under favorable conditions. These seed usually will not germinate under unfavorable germinating conditions. Seed with completely colorless cotyledonous tissue should not be considered germinable even though the essential structures stain a positive red. Examples of germinable and nongerminable dicotyledonous seed as indicated by tetrazolium tests are shown in Figure 11.

Tetrazolium tests not only estimate seed viability, but also reveal the relative level of vitality or seed vigor. A high percentage of the corn seed in Figure 12 will produce countable seedlings in germination tests; however, the stain test reveals various areas of internal seed weakness. These seed and similar seed of other crops will germinate satisfactorily under ideal conditions in the germinator, but often perform poorly in field tests or under adverse planting conditions in the

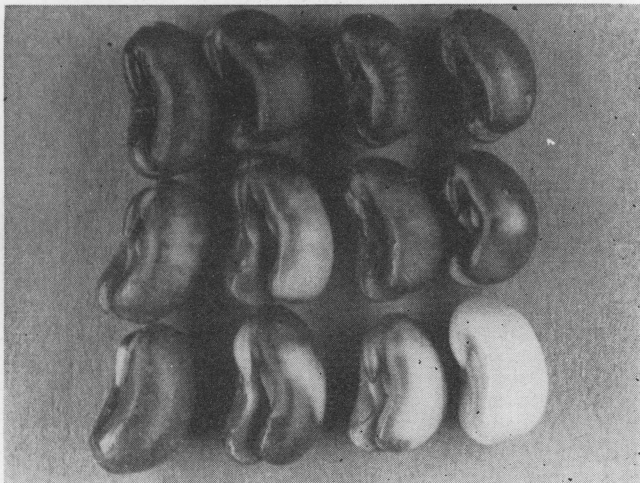


Figure 11. Dicotyledonous seed (field peas) exhibiting various staining patterns and levels of viability. Top row, seed capable of germination. Middle row, seed capable of germination, but low in vigor. Bottom row, nongerminable seed.

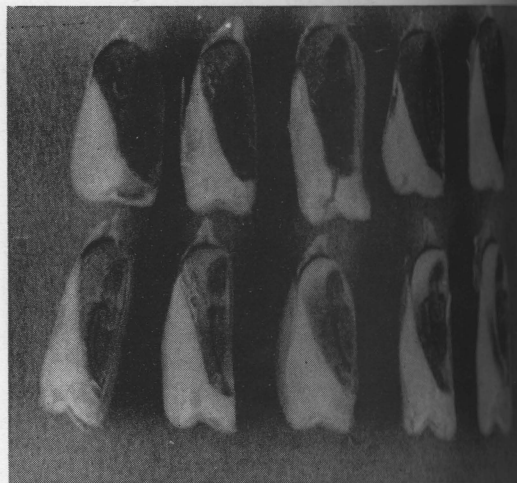


Figure 12. The vigor of corn seed revealed by tetrazolium test. Top row, a positive stain indicating vigorous seed. Bottom row, a cloudy, unclear stain indicating weakened embryo parts (low vigor). Note the colorless tissue in the lower scutellum.

field. The wide differences in germination results of these seed in laboratory tests and actual field emergence can be partly attributed to standard germination tests which fail to reveal seed with weakened embryo parts. The tetrazolium test allows the operator to evaluate critically the level of seed vigor by inspecting stained embryo parts. Although a faint red or an off-type stain coloration indicates low viable seed, the most common type of staining pattern exhibited by seed low in vigor is a mottled or lightly stained scutellum, while the plumule or primary root shows a light to positive red stain. However, if the scutellum is colorless, the seed should be considered ungerminable. Seed low in vigor stain more slowly than highly vigorous seed. Dicotyledonous seed low in vigor usually have various shades of

stain coloration in which the cotyledons are mottled, but the essential structures stain a positive red, Figure 11. Certain dicotyledonous seed such as cotton may stain an abnormally dark red color if extremely weak or aged.

Comparative Tetrazolium and Germination Tests of Various Crops

The data in Table 2 show the germination of duplicate seed samples of various crops by the tetrazolium stain and the standard germination methods. The comparative figures of each method represent the average percentage obtained in a number of samples from each crop.

Although the stain method revealed some weak seed in most samples, these seed were considered germinable if the vital embryo structure showed a positive red stain though such seed may not germinate satisfactorily under adverse field conditions. In general, the tetrazolium stain test provides a good guide for reporting the number of germinable seed, but the actual germination of some crops may be overestimated, especially if seed are dormant.

The wide difference in stain and germination results in the partially dormant seed in Table 2 was expected since this dormant condition has no apparent effect on the staining ability of the seed. Since the potential germination of dormant seed can be readily estimated with the tetrazolium test, this method often has a distinct advantage over the standard germination method with this particular seed. The data of both test methods conducted on samples of Rescue grass seed, *Bromus catharticus*, drawn from the same seed lot are presented in Table 2. Stain and germination test results indicate wide differences when partial dormancy exists, but when the dormancy is removed prior to testing, a close agreement was obtained between the two test methods. Further evidence of another type of dormancy is noted in clover varieties in which the hard seed fail to germinate, but the stain test tends to estimate the number of potentially germinable seed.

When the stain tests indicate that seed of a certain lot are slightly weakened, countable seedlings may or may not develop if such seed are germinated. These weakened seed are more sensitive than vigorous seed to any slight change in temperature of moisture conditions during the standard germination test. If seedling development is slow during the standard germination test, mold growth often prevents seedlings from obtaining countable size, although the vital embryo structures may show sufficient stain to be considered germinable by the tetrazolium test. These factors may account for some of the differences in results when determining germinable seed by both the tetrazolium and standard germination methods.

TABLE 2. COMPARATIVE TETRAZOLIUM STAIN AND GERMINATION TEST

Crop	Number samples tested ¹	Average percent germinable seed determined by tetrazolium stain reaction	Average percent normal seedling determined by germination test
Bean (single crosses)	15	92.8	88.7
Bean (inbreds)	5	95.0	92.5
Barium			
(pollinators)	8	64.1	55.3
Barium			
(US Katir-60)	4	95.0	89.0
Barley (Cordova)	3	95.0	94.5
Bast	9	93.0	89.3
Bean	10	86.0	90.0
Barley	3	90.0	92.0
Barn Millet	3	77.0	75.0
Barium			
(Rightmaster)	2	86.0	81.0
Barium (Braxos)	2	69.0	58.0
Barium			
(cream 12)	4	85.0	87.0
Barium	3	94.0	88.0
Barium (Hairy)	3	84.0	87.0 (Hard seed 8 percent)
Barium (Crimson)	2	100.0	95.0
Barium (Floranna)	2	85.0	78.0 (Hard seed 11 percent)
Barium (S-1 White)	2	95.0	76.0 (Hard seed 13 percent)
Barium (Indian)	2	82.0	73.0
Barium (Johnson)	2	61.0	55.0
Barium (Rescue)	2	85.0	56.0 (Partially dormant)
Barium "	2	86.0	83.0 (Nondormant)
Barium (Weeping Love)	2	82.0	86.0

¹ Each sample represents 100 seed drawn for tetrazolium stain and germination test, respectively. Wheat and oat samples include four different varieties in each crop.

The tetrazolium test has certain limitations that should be known to the operator. The standard germination tests are conducted according to the rules and regulations of the Association of Official Seed Analysts, but at the present time there are no universal rules and regulations for conducting the tetrazolium test. It may be some time before this method is standardized since more research is needed to determine the best method for conducting the test and interpreting the results. While the time required for staining the seed in a vitascope and interpreting the results is relatively short (10 to 50 minutes), the preparation of seed prior to staining often is tedious and requires considerable time, especially on small pasture grass seed. The tetrazolium test for estimating seed germination should not be considered a substitute for the standard germination test when the seedsman desires information to put on the Texas Tested Seed Label. It is not a substitute for all seed viability tests since more research is needed. The test is more expensive and requires a more skilled operator.

However, the number of individuals using the tetrazolium test may continue to increase rapidly in the next few years. This method now can be

very valuable to the larger seedsmen, seed analysts and managers of cotton delinting and other crop processing plants when they wish to obtain a quick fairly accurate estimate of the germination of seed. The more experienced operator usually can determine the relative vigor of seed and estimate fairly accurately how they will emerge under adverse field conditions. This test also is very valuable in determining the germination of dormant seed and in estimating the extent of mechanical injury to seed in harvesting and processing. The tetrazolium stain test now is a valuable tool for the seed industry and should be even more valuable in the future.

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